

The Role of Oxidative Stress and Antioxidant Supplementation in Pregnancy Disorders

Lucilla Poston¹, Natalia Igosheva¹, Hiten D. Mistry¹, Andrew H. Shennan¹, S Ananth Karumanchi², Lucy C. Chappell¹.

¹Division of Reproduction and Endocrinology, King's College London, Kings Health Partners Women's Health Clinical Academic Grouping

²Hughes Medical Institute, Center for Vascular Biology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Mass; Division of Epidemiology, Statistics

Address for correspondence

Professor Lucilla Poston, Maternal and Fetal Research Unit

10th floor North Wing, St.Thomas' Hospital, London SE1 7EH

Email lucilla.poston@kcl.ac.uk

Tel (44) 207 188 3639

Fax (44) 207 620 1227

Sources of Support; Tommy's the Baby Charity, The Wellcome Trust

Running Head: Oxidative Stress and Pregnancy Disorders

Abstract 250 words max

Oxidative stress is widely implicated in reproductive performance including infertility, miscarriage, diabetes-related congenital malformations and pre-eclampsia. Maternal obesity is a strong risk factor for pre-eclampsia, and recently, in an animal model of maternal obesity we have reported evidence of oxidative stress in the oocytes of obese animals prior to pregnancy as well as in early stage embryos. This adds to the growing evidence for a greater focus on the pre-conceptual period in prevention of pregnancy disorders including those related to oxidative stress. Our research has also focussed on the role of free radicals and antioxidant capacity in pre-eclampsia. Assessment by measurement of markers of lipid peroxidation or of antioxidant capacity has provided unequivocal evidence for oxidative stress in this disorder. Partial failure of the process of placentation has been implicated, with recent evidence proposing that ischaemia reperfusion in the placenta may contribute to oxidative stress in trophoblast. Endoplasmic reticulum stress in the placenta may also play a role. We and others have performed randomised controlled trials to determine whether early supplementation with vitamins C and E in women at risk of pre-eclampsia may be beneficial but these studies have shown no evidence for prevention of pre-eclampsia. Whether this represents an inappropriate antioxidant strategy or whether supplementation has been too late in gestation to be beneficial is not known. Other potential approaches to prevention of pre-eclampsia through amelioration of oxidative stress include provision of supplements in the pre-conceptual period, selenium supplements, anti-peroxynitrite strategies and statins.

Oxidative stress, defined as an imbalance between pro-oxidants and antioxidant capacity, has been implicated in sub-optimal reproductive performance from the earliest stages of development through to labour and delivery. Reactive oxygen species (ROS) are substances with one or more unpaired electrons; because of this ROS are highly reactive, interacting with lipids, proteins or DNA leading to oxidation and cellular malfunction which may initiate pathological processes. The most commonly produced of the ROS in mammals is superoxide. Depletion of antioxidant capacity, whether through low abundance of non-enzymatic (e.g. vitamins C, E, glutathione) or enzymatic (e.g. superoxide dismutase, glutathione peroxidases, catalase) antioxidants renders the cell vulnerable to oxidative attack, even under physiological situations where redox status is maintained through careful balance of a low level of synthesis of reactive oxygen species and the pathways of cellular defence (Raijmakers MTM et al, Current Pharm Design 2004; Forman HJ et al Biochemistry 2010).

Oxidative Stress and Fertility.

Gametes are vulnerable to oxidative attack (Ruder EH et al, Human Reproduction Update 2009; Aitkin RJ and Iuliis Mol Human Reproduction 2010). Reduced fertility in men has been associated with oxidative damage to sperm. Human sperm is vulnerable to oxidant attack as it contains a very high content of polyunsaturated fatty acids, which are necessary to facilitate fusion with the oocyte, but are rich in double bonds which are prone to oxidation. The spermatozoa of subfertile patients contain high levels of 8-hydroxy-20-deoxyguanosine (8OHdG), the oxidation product formed when DNA is subjected to attack by ROS (Kodama H et al., Fertil and

47 Sterility 1997). De Iuliis and colleagues have recently reported the presence of
48 8OHdG adducts in human spermatozoa to be highly correlated with DNA strand
49 breaks, determined using the TUNEL assay (De Iuliis et al., 2009). In a recent review
50 Aitken and De Iuliis list the possible mechanisms which may result in DNA strand
51 breaks in human sperm (Aitken and De Iuliis 2010). These include reduced
52 antioxidant capacity in epididymal plasma or seminal fluid (as may occur in
53 smokers), infection and iatrogenic ROS synthesis. These authors propose that the
54 latter is predominantly derived from mitochondria and may be exacerbated in
55 damaged sperm. It is suggested that damaged sperm, characterised by retention of
56 residual cytoplasm, abnormal chromatin remodelling or abnormally high content of
57 polyunsaturated fatty acids, will default to preferential activation of pathways of
58 apoptosis. This in turn will lead to excess mitochondrial ROS synthesis.
59 Mitochondrial oxidative phosphorylation necessarily leads to synthesis of free
60 radicals through electron 'leakage' from the electron transfer pathway leading to
61 generation of superoxide (O_2^\bullet) and hydroxyl radicals (OH^\bullet) and mitochondrial
62 activation in association with apoptosis can lead to excessive ROS synthesis and
63 'auto' attack of the already vulnerable sperm, DNA oxidation and subsequent
64 malfunction. Improved sperm function *in vitro* by addition of antioxidants has been
65 repeatedly shown (Baker HW et al Fertility and Sterility 1996; Donnelly ET et al,
66 Mutagenesis 2000) and it might be anticipated that antioxidant supplements would
67 improve fertility in sub-fertile men. Several studies suggest that antioxidant
68 supplementation may be of benefit in those subfertile men with proven oxidative
69 damage in the sperm but there is no consensus of opinion in regard to the
70 appropriate supplement or dose, and infertile men are not routinely tested for DNA
71 fragmentation (Deepinder F et al, Endocr Pract 2008). One study has reported the

potential benefit of antioxidant supplementation (1 g vitamin C and 1 g vitamin E daily for 2 months) in men with already proven high levels of DNA oxidative damage and one failed ICSI attempt. The majority of the men demonstrated a reduction in DNA fragmented spermatozoa and improved ICSI success (Greco E, Human Reproduction 2005). An improvement in fertility was also found in men with a high DNA fragmentation index instructed to consume a diet rich in antioxidants or commercial multivitamins containing beta-carotene, vitamin C, vitamin E, and zinc for at least 3 months (Gill Villa AM Fertility and Sterility 2009). Our group have recently systematically reviewed the effect of oral antioxidants on male subfertility and concluded that supplementation could improve sperm quality and/or pregnancy rates; however large adequately powered trials using individual antioxidants are required (Ross et al., 2010).

Oxidative stress also influences fertility in species other than man. An interesting corollary from the animal kingdom, recently published, is the recognition that amongst males of several avian species, those which are more brightly coloured are the more fertile. In the Great Tit (*Parus major*), the intensity of the yellow colour of the male breast has been related to the degree of carotenoid sufficiency, which in turn has been found to protect the sperm from lipid peroxidation, and thereby improve chances of reproductive success (Helfenstein F et al Eco Letters 2010).

Oocyte quality is also affected by oxidative stress, and lower fertility rates in cigarette smokers, or in association with high levels of alcohol consumption, have been have been linked to increased ROS synthesis (Paszkowski T et al, Clin Chim Acta 236; 1995; Jensen TK et al, BMJ 1998;317; Eggert J et al, Fertil Steril 2004; 81; Ruder

EH et al 2008). As in many cellular processes, a low level of ROS synthesis fulfils an important role in cell signal transduction pathways, and in the oocyte, is a prerequisite for the first meiotic phase (MI) and also a requirement for folliculogenesis. However excessive ROS synthesis will impair oocyte maturation (MII) and inadequate intracellular antioxidant capacity, particularly low concentrations of reduced glutathione (GSH), can limit successful ovulation and fertilisation (Ruder et al, 2008). Whilst there is good evidence that dietary antioxidant supplements can modulate fertility in rodents, there is as yet very limited evidence to suggest that periconceptional antioxidant supplementation should be recommended to improve fertility in women (Ruder et al, 2008; Cetin I et al, Human Reprod Update 16; 2010).

In vitro fertilization is also affected by excessive ROS in the embryo culture media, and the routine practice of incubation in a low oxygen tension prevents embryo arrest and enhances the chance of successful fertilization. (Ruder et al; 2008)

Obese women have a high rate of infertility, and assisted conception is often associated with a low success of oocyte fertilization, or of failure of embryo development (Pandey S and Battacharya S; Womens Health 2010). Several biomarkers of oxidative stress are increased in the blood of obese non-pregnant and pregnant individuals (Iyer A et al Nature Rev Endocrinol 2010; Jarvie E et al, Clin Science 2010). In a recent study we addressed the hypothesis that oxidative stress may be a contributory factor to reduced fertility in obese pregnancies (Igosheva N et al, PlosOne 2010). We explored the hypothesis that increased substrate availability

121 for mitochondrial respiration may lead to oxidative stress in the oocyte and
122 developing embryo. It is proposed that a high plane of nutrition may lead to
123 excessive enrichment of the reproductive milieu (Robker RL et al, J Clin Endocrinol
124 Metab 2009) and high rates of metabolism may compromise oocyte and embryo
125 development, potentially through excessive mitochondrial ROS synthesis. We
126 determined whether obesity in the mouse is associated with increased mitochondrial
127 activity in oocytes and early stage embryos. C67BL/6J mice were fed a highly
128 palatable diet or normal laboratory chow. After six weeks of the diet, females were
129 induced to superovulate by hCG and oocytes were collected by puncture of pre-
130 ovulatory follicles. Zygotes and blastocysts were collected after successful mating
131 with lean males after 24 and 84hr post hCG. Mitochondrial membrane potential, an
132 indirect measure of mitochondrial activity, was determined with a mitochondrial
133 specific membrane fluoroprobe (TMRM). Hyperpolarization of the membrane was
134 observed in oocytes and zygotes retrieved from the obese females when compared
135 to those from the lean animals. Redox status was assessed by measurement of the
136 oxidative status of the pyridine nucleotide (NAD(P)H) and the flavine nucleotide
137 FAD⁺⁺, by measurement of autofluorescence. Both showed evidence of increased
138 oxidation in oocytes and zygotes from the obese females. Direct measurement of
139 free radical generation in *in vitro* 'real time' using the fluorescent dye dihydroethidium
140 (HEt) also showed clearly that obesity was associated with increased ROS
141 synthesis. Measurement of reduced glutathione using the fluorescent dye
142 monochlorobimane (MCB) provided evidence of reduced cellular antioxidant
143 capacity. ROS can also affect mitochondrial abundance and copy number and we
144 found in the oocytes that mitochondrial DNA copy number was increased together
145 with expression of nuclear genes encoding mitochondrial DNA transcription factors

(mtTFAM and NRF1). The ability of zygotes to develop to the blastocyst stage was also reduced in the obese mice. To our knowledge this is the only study to have directly addressed redox status in oocytes and early embryos in obese animals and supports the hypothesis that oxidative stress may play a role in suboptimal fertility in obesity.

Using the same model of murine obesity our laboratory also reported previously that the offspring later become hypertensive, demonstrate glucose intolerance and are fatter than controls (Samuelsson et al, Hypertension 2008). We also reported that the adult (3 month old) male offspring of the obese dams display a decrease in the mitochondrial electron transport chain as shown by reduced mitochondrial-linked complex II-III (Shelley, McConnett et al, Am J Physiol 2009). Thus abnormal mitochondrial function is an accompaniment of obesity from the earliest stages of life. Whether this played a role in development of the phenotype described in the adult offspring of the obese dams, or whether it was a consequence remains to be determined but there is growing evidence that the mitochondrion, which itself is particularly susceptible to DNA damage and has a modest capacity for repair, may be involved in the epigenetic processes associated with the developmental of adulthood disorders arising from nutritional imbalance *in utero* and in early post natal life (Simmons RA Rev End Met Dis 2007). **Figure?**

Oxidative Stress and Early Pregnancy Loss.

Oxidative stress has also been implicated in early miscarriage. Jauniaux and Burton have suggested that miscarriage may arise from premature oxygenation of the early embryonic environment. Using an O₂ probe in women prior to first trimester

termination they showed a steep rise in placental pO₂ between 8 and 12 weeks of gestation, coincident with the establishment of maternal perfusion. Oxygenation was accompanied by upregulation of a battery of antioxidant defences including increased expression of catalase, glutathione peroxidase and Cu/Zn and Mn superoxide dismutase (Jauniaux E et al, Am J Pathol 2000). The same group showed an increase in markers of oxidative stress in placental tissue from early pregnancy losses compared with controls and suggested that increased ROS generation may arise from a consequence of the premature establishment of maternal placental perfusion (Burton G, Jauniaux E, J Soc Gynecol Invest 2004). Whilst it might be anticipated that antioxidant supplements may provide some protection against miscarriage, metanalysis of all relevant studies suggests no substantive evidence for antioxidant supplements providing any benefit (Rumbold A, Middleton, Crowther, Cochrane Review 2005).

Oxidative Stress and Pre-eclampsia.

Pre-eclampsia, which affects approximately 2-7% of all pregnancies, is a syndrome associated with multi-organ dysfunction, characterised by new onset hypertension (blood pressure $\geq 140/90$ mmHg) and proteinuria (≥ 300 mg/L) after 20 weeks' gestation (Brown et al., 2001). Other complications including stroke, convulsions, pulmonary edema, liver failure and thrombus formation make this a potentially life threatening condition for the mother and child (Villar J, Say L, Gulmezoglu AM et al. Eclampsia and pre-eclampsia: a worldwide health problem for 2000 years. In Critchley H, MacLean A, Poston L, Walker J '*Pre-eclampsia*'. London (UK) RCOG Press 2003; 189-207). Infant mortality and morbidity may also be may compromised

by placental insufficiency leading to poor fetal growth. The precise mechanisms that lead to pre-eclampsia, which often occurs without warning and may follow a precipitous course are not known, but failure of the normal processes of placentation, followed by inadequate placental perfusion would seem to be a necessary prelude to the cascade of molecular events which culminate in the maternal syndrome (Redman CWG and Sargent IL, Placental Stress and Pre-eclampsia, Placenta 2009). This is characterised by a marked exaggeration of the normal, mild inflammatory response which occurs in the pregnant woman, and is accompanied by vascular endothelial cell and platelet activation, and impairment of vascular endothelial dilator function (Redman CW, [Sargent IL](#). Preeclampsia and the systemic inflammatory response. [Semin Nephrol](#). 2004 Nov;24(6):565-70).

The normal process of placentation involves remodelling of the maternal spiral arteries, the small resistance arteries of the uterine circulation responsible for blood supply to the placental intervillous space. The normally thick muscular wall of the spiral artery is rendered flaccid and non-contractile as the cytotrophoblast (placental epithelial cells) invade the decidua and myometrium of the uterine wall (Pijnenborg et al., 1980; Jauniaux et al., 2006). In pre-eclampsia this process is often incomplete and some vessels retain the smooth muscle layer ([Whitley GS](#), Cartwright JE. Cellular and Molecular Regulation of Spiral Artery Remodelling: Lessons From the Cardiovascular Field. [Placenta](#). 2010 Mar 30; doi:10.1016/j.placenta.2010.03.002). This in turn leads to reduced placental perfusion. The resultant hypoxia, together with intermittent reperfusion, is hypothesised to provoke ROS synthesis in the placenta (Burton, Hwang, Cindrova Davies, Placenta 2009; 23; S43). Numerous

218 studies describe elevation of markers of oxidative stress in placental tissue from
219 women with pre-eclampsia (Raijmakers, 2004 Current Pharmaceutical Desgin;
220 Burton, Hwang etc 2009). Periods of ischaemia followed by reperfusion are
221 associated with conversion of xanthine dehydrogenase to xanthine oxidase which is
222 a potent source of superoxide (O_2^{\bullet}) and xanthine oxidase activity is increased in the
223 placentae of affected women (Many A, Hubel CA, Fisher SJ, Roberts JM, Zhou Y.
224 Invasive cytotrophoblasts manifest evidence of oxidative stress in preeclampsia. Am
225 J Pathol. 2000 Jan;156(1):321-31). Recently, Burton *et al* have shown that hypoxia
226 in placental tissue leads to endoplasmic reticulum (ER) stress, and activation of the
227 unfolded protein response (UPR) (Burton GJ, H-W Yung Placenta 2009; 23; S43).
228 ER stress leads to accumulation of misfolded proteins, which is the trigger for the
229 unfolded protein response (UPR) that aims to restore ER homeostatic balance.
230 Failure of this mechanism, which attempts to reconfigure the folding of proteins can
231 lead to activation of apoptotic pathways and protein synthesis inhibition, which have
232 been implicated by Burton and colleagues in the pathway to fetal growth restriction.
233 Moreover ER stress is coupled to ROS synthesis, and ROS synthesis will increase
234 with the degree of maternal spiral artery malfunction (Burton GJ, H-W Yung Placenta
235 2009; 23; S43). Together ER stress and ROS synthesis are proposed to activate a
236 cascade of pathways leading to cytokine release, prostaglandin synthesis, increased
237 expression of anti-angiogenic factors (e.g. soluble Flt-1) and activation of apoptotic
238 pathways. Other suggested contributors to ROS production include an activating
239 autoantibody to the angiotensin 2 (ATII) receptor which is proposed to lead to ROS
240 synthesis through activation of NADPH oxidase, a key cellular source of superoxide
241 (Siddiqui AH, Irani RA, Blackwell SC, Ramin SM, Kellems RE, Xia Y. Angiotensin
242 receptor agonistic autoantibody is highly prevalent in preeclampsia: correlation with

disease severity. Hypertension. 2010 Feb;55(2):386-93). As summarised in figure 1, it is hypothesised that these disturbances of placental function may be causative of the maternal syndrome.

Antioxidant Supplementation in Pre-eclampsia

The recognition of oxidative stress in the placenta, and also in the maternal circulation, prompted us to evaluate the potential benefit of prophylactic antioxidant supplementation in women with known risk of pre-eclampsia. At first we evaluated the effect of vitamin E and C supplements in women with known risk factors for pre-eclampsia (abnormal uterine artery Doppler waveform or previous pre-eclampsia) (Chappell LC, Seed PT, Briley AL, Kelly FJ, Lee R, Hunt BJ, Parmar K, Bewley SJ, Shennan AH, Steer PJ, Poston L. Effect of antioxidants on the occurrence of pre-eclampsia in women at increased risk: a randomised trial. Lancet. 1999 Sep 4;354(9181):810-6). The study was designed to test the hypothesis that antioxidants would lead to reduction of biomarkers of maternal endothelial dysfunction, the ratio of Plasminogen Activator Inhibitor (PAI)-1: PAI-2 (PAI-1:PAI-2) being chosen as the primary outcome. In this small randomised controlled trial of 283 women, we showed that supplementation with 1gm vitamin C and 400IU of vitamin E daily from around 16 weeks' of gestation until delivery was associated with significant reduction in the PAI-1:PAI-2 ratio. We also reported a reduction in the plasma concentration of 8-epi prostaglandin F_{2α}, a marker of lipid peroxidation, in association with elevation of the plasma vitamin C and E concentrations (Chappell LC, Seed PT, Kelly FJ, Briley A, Hunt BJ, Charnock-Jones DS, Mallet A, Poston L. Vitamin C and E supplementation in women at risk of preeclampsia is associated with changes in indices of oxidative

267 stress and placental function. *Am J Obstet Gynecol.* 2002 Sep;187(3):777-84).

268 Although not powered for pregnancy outcome the number of women who developed

269 pre-eclampsia was lower in the antioxidant group compared with those women

270 taking the placebo preparation. Encouraged by the evidence that antioxidants could

271 improve oxidative stress and by the suggestion of reduced occurrence of the

272 disease, we performed a randomised controlled trial in 2404 women, adequately

273 powered to detect a difference in the incidence of pre-eclampsia, the primary

274 outcome (Poston L, Briley AL, Seed PT, Kelly FJ, Shennan AH; Vitamins in Pre-

275 eclampsia (VIP) Trial Consortium. Vitamin C and vitamin E in pregnant women at

276 risk for pre-eclampsia (VIP trial): randomised placebo-controlled trial. *Lancet.* 2006

277 Apr 8;367(9517):1145-54). Although treatment compliance was good and despite

278 evidence for improved antioxidant capacity in the blood, there was no difference in

279 the number of women who developed pre-eclampsia between intervention (15%)

280 and placebo (16%) arms. There was also a small but statistically significant increase

281 in the incidence of low birthweight in the intervention arm (Risk Ratio 1.15 (1.02 to

282 1.30). Three other large randomised controlled trials including one undertaken by the

283 WHO in developing countries have also shown a lack of effect, conclusively proving

284 that this antioxidant regime does not prevent pre-eclampsia either in high risk (Villar

285 J, Purwar M, Merialdi M, Zavaleta N, Thi Nhu Ngoc N, Anthony J, De Greeff A,

286 Poston L, Shennan A; WHO Vitamin C and Vitamin E trial group. World Health

287 Organisation multicentre randomised trial of supplementation with vitamins C and E

288 among pregnant women at high risk for pre-eclampsia in populations of low

289 nutritional status from developing countries. *BJOG.* 2009 May;116(6):780-8) or lower

290 risk (Rumbold AR, Crowther CA, Haslam RR, Dekker GA, Robinson JS; ACTS

291 Study Group. Vitamins C and E and the risks of preeclampsia and perinatal

complications. N Engl J Med. 2006 Apr 27;354(17):1796-806; Roberts JM, Myatt L, Spong CY, Thom EA, Hauth JC, Leveno KJ, Pearson GD, Wapner RJ, Varner MW, Thorp JM Jr, Mercer BM, Peaceman AM, Ramin SM, Carpenter MW, Samuels P, Sciscione A, Harper M, Smith WJ, Saade G, Sorokin Y, Anderson GB; Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Vitamins C and E to prevent complications of pregnancy-associated hypertension. N Engl J Med. 2010 Apr 8;362(14):1282-91) women. Two of these have also shown an increase in the incidence of gestational hypertension in the intervention arm (Poston; Roberts), although the observation of low birthweight found in our RCT has not been replicated in the subsequent trials.

Why do antioxidants Vitamin C and E not prevent Pre-eclampsia?

Despite overwhelming evidence for oxidative stress in pre-eclampsia, a regime of a vitamin C and E supplementation does not prevent pre-eclampsia. Amongst the several potential explanations, the first must be that oxidative stress, whilst undoubtedly present, plays no causative role in the aetiology of the disorder. This counters all the observational studies and extensive *in vitro* analyses detailing the responsible signalling pathways potentially involved. Until evidence to the contrary is presented, this explanation cannot be discounted. The second is that this antioxidant regime is inappropriate. Longitudinal blood sampling was performed in a sub-group of participants in the second trial of vitamins C and E from our group; supplementation leads to a significant fall in the plasma concentrations of γ -tocopherol (Figure 2). The vitamin E preparation was given as natural source RRR α -tocopherol, the stereoisomer that is preferentially absorbed in humans. The fall in γ -

316 tocopherol might be anticipated since dietary α and γ tocopherol compete in the liver
317 for the tocopherol transfer protein (TTP) which facilitates uptake into the circulation
318 (Hosomi A, Arita M, Sato Y, Kiyose C, Ueda T, Igarashi O, Arai H, Inoue K. Affinity
319 for alpha-tocopherol transfer protein as a determinant of the biological activities of
320 vitamin E analogs. FEBS Lett. 1997 Jun 2;409(1):105-8). However γ -tocopherol has
321 biological activity, including anti-inflammatory properties; lowering of the blood
322 concentration may impair the capacity to combat the inflammatory response of pre-
323 eclampsia (Devaraj S, Jialal I. Failure of vitamin E in clinical trials: is gamma-
324 tocopherol the answer? Nutr Rev. 2005 Aug;63(8):290-3; Reiter E, Jiang Q, Christen
325 S. Anti-inflammatory properties of alpha- and gamma-tocopherol. Mol Aspects Med.
326 2007 Oct-Dec;28(5-6):668-91). It was of interest that antioxidant supplementation
327 also led to a significant fall in the plasma concentration of sflt-1, the anti-angiogenic
328 soluble receptor for VEGF-1 which has been strongly implicated in development of
329 pre-eclampsia, and a rise in placenta growth factor (PIGF) (Figure 3), but had no
330 effect on endoglin. Studies *in vitro* have also shown that vitamins C and E prevent
331 elevation of sFlt-1 in response to hypoxia- reperfusion in isolated placental
332 trophoblast from normal pregnancies (Cindrova-Davies et al, Gabor Lecture Award
333 Placenta 2009).

334
335 It is also possible that the randomised controlled trials of antioxidants have provided
336 the antioxidant supplements at too late a stage in gestation. Although the recently
337 reported study from the USA started antioxidants earlier in pregnancy than the other
338 trials (9-16 weeks' gestation) (Roberts et al 2010), there has been no study to
339 address prophylaxis over the periconceptual period. It is of interest that in a large

observational study in USA women, Bodnar et al have observed that regular use of multivitamin preparations in the peri-conceptual period was associated with a 45% reduction in pre-eclampsia risk compared with non-use (odds ratio 0.32-0.95) (Bodnar L et al. Am J Epidemiol 2006).

Alternative Strategies?

Vitamins C and E have little influence on the development of the peroxynitrite radical (ONOO•) formed from the interaction of nitric oxide (NO•, a nitrogen radical) with superoxide (O₂•). In a recent study, Davidge's group showed evidence for up-regulation of the LOX-1 receptor in the endothelium of small arteries dissected from omental biopsies obtained during Caesarean section from women with pre-eclampsia (LOX-1 is the receptor for oxidised LDL), which on gaining access to the endothelium leads to an inflammatory response, stimulating macrophage transudation across the endothelial barrier; LOX-1 expression is stimulated by peroxynitrite and these authors showed *in vitro* that a peroxynitrite scavenger prevented upregulation of LOX-1 by pre-eclamptic serum in arteries from normal pregnant women (Sankaralingam S et al, Hypertension 2009). Thus anti-peroxynitrite strategies may be an alternative evidence-based strategy for reducing oxidative stress and improving endothelial function in affected women. Here, melatonin may be a potential candidate as it is a recognised scavenger of ONOO•,

There is also a recognised association between essential micronutrient selenium status and pre-eclampsia, which may have implications for a different approach to

prevention of oxidative stress. Several selenoproteins, notable the glutathione peroxidases, play an important role in cellular antioxidant defence by reducing lipid hydroperoxides to their corresponding un-reactive alcohols and reducing free hydrogen peroxide to water (Oster & Prellwitz, 1990; Rayman, 2000). Geographically, there are wide variations in dietary selenium intake depending on the selenium content of the soil. Pre-eclampsia has been linked to lower placental tissue, blood and toenail (long term status) selenium status and to reduced activity of glutathione peroxidases (Mistry et al 2008; Atamer et al, 2005; Rayman et al., 2003). Our group recently reported increased plasma concentrations of thiobarbituric acid reactive substances (TBARS; a marker for lipid peroxidation) in maternal and cord plasma in women with pre-eclampsia compared to controls; moreover, total glutathione peroxidase activity in both maternal and cord plasma and in placental tissue was significantly reduced and plasma activity positively related to the plasma selenium concentration (Mistry, et al. 2008). Selenium supplementation therefore offers another potential strategy for pre-eclampsia prevention particularly in geographical regions such as Europe with low soil selenium content (Thomson, 2004). Further prospective, longitudinal studies are required to elucidate a 'cause or effect' relationship. A small randomised control trial of selenium supplementation (selenium in pregnancy; SPRINT) conducted in the UK is underway to assess the impact of selenium supplements on pre-eclampsia related biomarkers. Perhaps unsurprisingly, dietary selenium insufficiency has been linked to infertility through oxidative stress.

386 Theoretically, statins could play a role in prevention of pre-eclampsia, although
387 safety in pregnancy must first be established. Statins offer a multi-pronged rationale
388 as aside from improving the HDL:LDL cholesterol ratio statins have anti-
389 inflammatory properties, as well as antioxidant effects through inhibition of NADPH
390 oxidase (Montecucco F, Mach F. Update on statin-mediated anti-inflammatory
391 activities in atherosclerosis. *Semin Immunopathol.* 2009 Jun;31(1):127-42).

392
393 In summary, oxidative stress is associated with infertility, miscarriage and pre-
394 eclampsia, but there is at present little convincing evidence that antioxidant
395 supplements can improve fertility, or prevent miscarriage or pre-eclampsia. The
396 breadth of strategies attempted has not been extensive, and there is a good
397 evidence base to indicate well conducted, adequately powered trials which utilise
398 other approaches. Safety and ethical considerations must however remain a
399 predominant issue in any move towards other approaches.

Figure XX: Plasma g-tocopherol concentration across gestation in 89 women on placebo and 95 women taking vitamin C and E supplementation; effect of supplementation: 0.49 (CI 0.43 – 0.57).

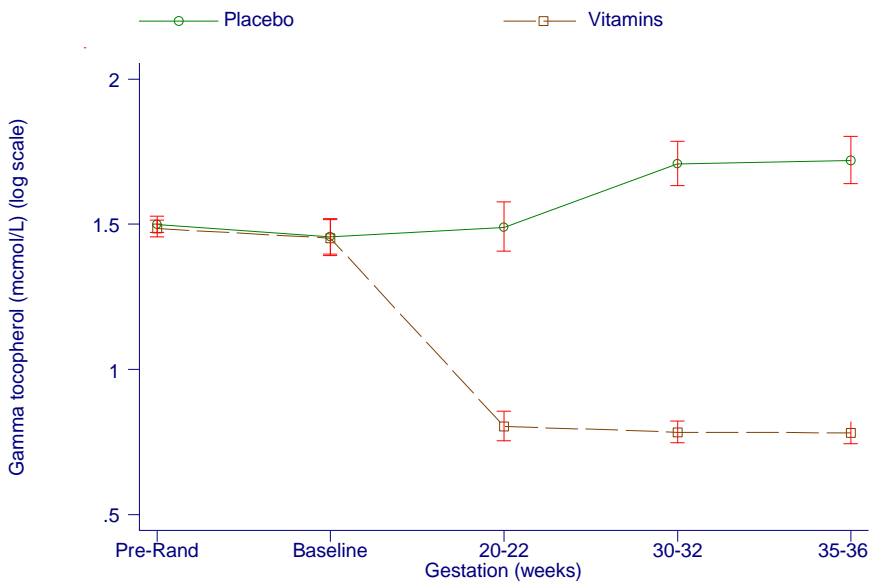


Figure XX: Plasma s-flt-1 concentration (logarithmic scale) across gestation in 45 women on placebo and 54 women taking vitamin C and E supplementation; effect of supplementation: 0.48 (CI 0.19 to 0.67 over 16 weeks), $p=0.037$, compared to the placebo group.



Figure XX: Plasma PIGF concentration (logarithmic scale) across gestation in 45 women on placebo and 54 women taking vitamin C and E supplementation; effect of supplementation: 2.1 (CI 1.03 to 4.40 over 16 weeks), $p=0.037$, compared to the placebo group.

